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# Lignification and Cell Wall Thickening in Nodes of *Phyllostachys viridiglaucescens* and *Phyllostachys nigra*

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• *Background and Aims* Bamboos are among the most important plants in the world. The anatomical structure and mechanical properties of the culm internode are well documented. Fewer details are available of the culm node. The aim of this study was a topochemical investigation on lignification and cell wall thickening in developing and maturing bamboo nodes. The deposition sequence and distribution of lignin structural units and cell wall thickening in different anatomical regions of the node of *Phyllostachys viridiglaucescens* and *Phyllostachys nigra* are discussed.
• *Methods* Cell wall thickening and lignification are investigated in the outer part of the nodal region and in the diaphragm of developing and maturing *P. nigra* culms and in maturing culms of *P. viridiglaucescens* of different age classes. The lignification during ageing was studied topochemically by means of cellular UV microspectrophotometry. A combination of light microscopy and image analysis techniques were used to measure cell wall thickness.
• *Key Results* The fibre and parenchyma cell wall thickness does not significantly increase during ageing. In the diaphragm, the cell walls are thinner and the cell diameter is larger than in the outer part of the node. In shoots, the lignin content in the epidermis, hypodermis and in both fibre and parenchyma cells of the diaphragm is relatively low compared with older culms. The fibre and parenchyma cells of the diaphragm have higher values of *p*-coumaric and ferulic acids than fibre and parenchyma cells of the outer part of the node.
• *Conclusions* It was hypothesized that the combination of more hydroxycinnamic acids and of thinner cell walls in combination with higher cell diameters (lower density and lower stiffness) in the diaphragm than in the outer part of the node may play an important role in the biomechanical function of the node by acting as a spring-like joint to support the culm by bending forces.

**Key words:** Bamboo, *Phyllostachys viridiglaucescens*, *Phyllostachys nigra*, nodes, anatomy, lignification, cell wall thickening, UV-microspectophotometry.

## INTRODUCTION

Because of their economic importance and multiple uses in daily life bamboo culms and more specifically bamboo internodes have been investigated thoroughly. In contrast to the rather straightforward anatomy of the internodes, the composition and structural details of the more complex nodes have not been analysed as much (Zee, 1974; Liese and Ding, 1994; Ding and Liese, 1995, 1997; André, 1998). Nevertheless, the nodes have special significance for culm function. Owing to the lack of radial conduction cells, they enable the necessary communication for cross-transport of both water and nutrients. Furthermore, the nodal structure is important for liquid movement during drying and preservation as well as for physical and mechanical properties of the culm. The functional role of nodal diaphragms is to act as braces to resist wall invagination or buckling (Niklas, 1997, 1998). Because nodal diaphragms brace internodal walls against lateral contraction, they increase the effective stiffness of stems. They also function as spring-like joints that store energy to elastically restore stems to their original shapes when bending forces are removed (Niklas, 1997, 1998). In internodes, cell wall thickening and lignification is considered to take place over several years (Alvin and Murphy, 1988; Majima *et al*., 1991; Liese and Weiner, 1996, 1997; Murphy and Alvin, 1997*a*, *b*; Gritsch *et al*., 2004). This ability to prolong cell wall thickening of fibres and parenchyma cells may provide an excellent mechanism for further strengthening the culm as it ages (Murphy and Alvin, 1997*a*). Even though the importance of cell wall thickening and lignification for mechanical strength in the culm has been investigated, it has never been studied in detail in the nodes of bamboo.

Within the framework of research on changes related to ageing of bamboo culms, the complex anatomical structure of the node was studied with emphasis on its topochemical distribution of lignin and cell wall thickening.

## MATERIALS AND METHODS

### *Bamboo samples*

Samples of the nodal area and of the culm sheath were taken from shoots of *Phyllostachys nigra* (Loddiges ex Lindley) Munro growing in the University Botanical Garden of Ghent, Belgium (Table 1). Older culms of *Phyllostachys nigra* were sampled both in the University

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TABLE 1. *Samples used for this study*

| Species | Origin | Age (in months) | Remarks |
|---|---|---|---|
| *P. nigra* | Ghent | Shoot I | 12 cm height, completely surrounded by culm sheaths |
| | | Shoot II | 360 cm height,<br>Nodes 1–6*: culm sheaths not present;<br>Nodes 7–15: surrounded by culm sheaths, internode elongated;<br>nodes 15–top*: completely surrounded by culm sheaths |
| | | 1, 3, 6, 9, 12 | |
| | Prafrance | 8, 20, 32, 44, 56, 68, 80, 92, 104 | |
| *P. viridiglaucescens* | Meise | 1, 3, 6*, 9, 12* | |
| | Prafrance | 8, 20, 32, 44, 56, 68, 80, 92, 104* | |

The shoots of *P. nigra* were used to study the structure of the nodes and early lignification.
Samples marked by * were used for UV-microspectrophotometry.

Botanical Garden of Ghent, Belgium (1, 3, 6, 9 and 12 months old) and at the Bambuseraie in Prafrance (France) (8, 20, 32, 44, 56, 68, 80 and 104 months old) (Table 1).

Bamboo culms of the species *Phyllostachys viridiglaucescens* (Carr.) Riv. & Riv (1, 3, 6, 9 and 12 months old) were harvested in the National Botanical Garden of Belgium (Meise). Older culms (8, 20, 32, 44, 56, 68, 80 and 104 months old) were sampled at the Bambuseraie in Prafrance (France). Blocks of the nodal area between the 6th and 7th internode (numbered from ground level) were cut and preserved in a mixture of 50 % alcohol, 10 % glycerine and 40 % water.

### *Microscopy and cell wall measurements*

Young, soft nodes and culm sheaths of *P. nigra* shoots were dehydrated in a graded series of alcohol and embedded in Technovit 7100 (Heraeus Kulzer, Wehrheim, Germany). Transverse and longitudinal sections (5 µm thick) were cut using a rotary microtome (Minot-Mikrotom1212, Leitz, Wetzlar, Germany) and stained with toluidine blue O. Toluidine blue O stains lignified cell walls blue-green and unlignified cell walls reddish purple (O'Brien *et al*., 1964). The sections were permanently mounted in DePeX (Merck, Lutterworth, UK).

From the older samples, transverse and longitudinal sections (18–20 µm thick) were cut using a sliding microtome (Microm HM440E) and double stained with safranin and astrablue. After dehydration and clearing in Parasolve (Prosan 88001-0, Ghent, Belgium), the sections were permanently mounted in Entellan (Merck, Darmstadt, Germany).

All observations were made on an AHBS-21 Vanox Olympus universal microscope using bright field illumination. For the cell wall measurements the image analysis program analySIS 3·0 (Soft Imaging System) was used. Fibre wall thickness was measured in the middle of the outer culm wall and both fibre and parenchyma wall thicknesses were measured in the diaphragm. For each characteristic, 50 measurements were recorded on transverse-sectioned cells.

### *UV-microspectrophotometry*

Small blocks ($1 \times 1 \times 5\,mm^3$) were cut from selected material of *P. nigra* and *P. viridiglaucescens* (Table 1; *P. nigra* shoot II: node between 2nd and 3rd and between 18th and 19th internode; *P. viridiglaucescens*: node between 6th and 7th internode). These specimens were dehydrated in a graded series of acetone and impregnated with Spurr's resin (Spurr, 1969) through a series of propylenoxide/spurr resin mixture, followed by immersion in pure resin.

Transverse sections through the node (1 µm in thick) were cut with an ultramicrotome using a diamond knife. The sections were transferred to quartz microscope slides, immersed in a drop of non-UV absorbing glycerine and covered with a quartz cover slip. The sections were observed using the immersion ultrafluar objective 32 : 1. The immersion oil used consisted of a glycerine/water mixture $n_D = 1{\cdot}46$.

The sections were examined under a ZEISS UMSP 80 microspectrophotometer equipped with a scanning stage enabling the determination of image profiles at defined wavelengths. The specimens were investigated by point measurements with a spot size of $1\,\mu m^2$ between 240 and 400 nm wavelengths using the program LAMWIN® (Zeiss). $S_2$ wall layers of epidermis, hypodermis, fibre and parenchyma cells, the compound middle lamellae and the vessel cell wall were measured at the outer culm wall and at the diaphragm.

Image scan profiles at a constant wavelength of 280 nm were generated using the scan program APAMOS® (Zeiss). This program digitizes rectangular fields of the tissue with a geometrical resolution of $0{\cdot}25\,\mu m^2$ and a photometrical resolution of 4096 grey-scale levels, which are converted into 14 basic colours to visualize the absorbance intensities.

## RESULTS

In the following description, the morphological terms as defined by McClure (1966) are used. The sheath scar is called the nodal ridge and the bulge formed by the intercalary meristem the supranodal ridge (Fig. 1).

### *Structure of the node*

During culm growth, an internodal central cavity called the lacuna is formed. In young, still elongating shoots, this central part consists of parenchyma cells that will die and

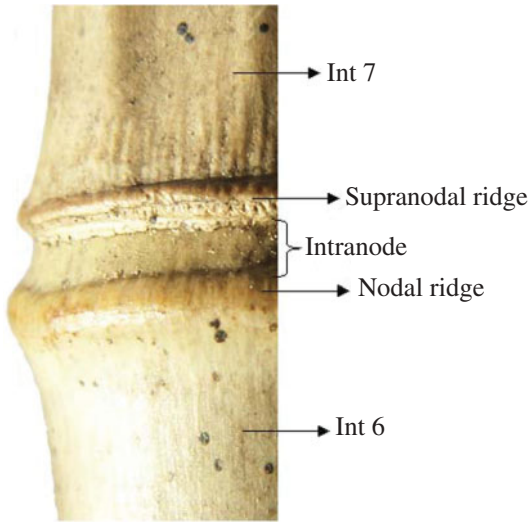



FIG. 1. Illustration of a node of *Phyllostachys nigra*.

disappear upon completed elongation. Figure 2A shows a longitudinal section through the diaphragm of a node (shoot II, between internodes 6 and 7) of an almost completely elongated part of the culm. The parenchyma cells adjacent to the parenchyma cells, which will die, become sclerified. These cells will form the border between the diaphragm and the lacuna. In the node, the normal arrangement of the vascular bundles with four fibre caps vanishes and a sclerenchyma sheath can be observed. Some bundles have only one vessel element. In the same section, both longitudinally and transversely sectioned vascular bundles are present, indicating that they run criss-cross in the nodal structure. Branching vascular 'anastomoses' are developed as can be seen in Fig. 2. The branching of phloem and xylem does not always start simultaneously. The fibres and parenchyma cells in the diaphragm have an irregular form.

Figure 2B and C illustrates that cell wall lignification proceeds from the bottom towards the top of the internode. Figure 2B shows a longitudinal section through a node between the 2nd and the 3rd internode. In comparison to a longitudinal section through a node between the 18th and the 19th internode (Fig. 2C), the cell walls are blue coloured (staining with toluidine blue) which indicates higher lignin content.

At the nodal ridge the vascular bundles mostly have two fibre caps, one at the xylem and one at the phloem side. Ramification has already begun in this portion. At the supranodal ridge, the vascular bundles have a different form. The fibre caps are larger at the phloem and protoxylem side and smaller at the metaxylem side. More hypodermis cells are present compared with the number of cells in the internodes.

The culm sheath has a hypodermis with thick-walled, lignified cells. The inner cells opposite the vascular bundle are also sclerified. So, the culm sheath forms a hard structure that protects the underlying still-developing culm.

*Fibre and parenchyma wall thickness in the nodal structure*

Figure 3A shows mean cell wall thickness of fibre and parenchyma cells in the nodal structure of *P. nigra* culms.

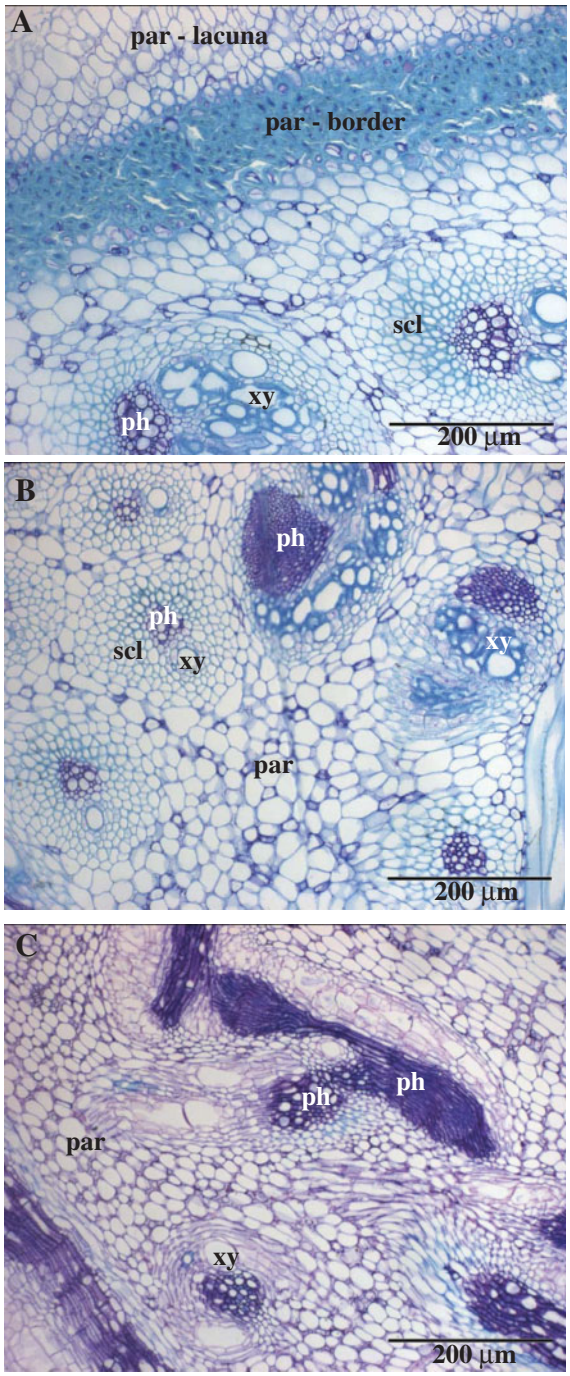



FIG. 2. Longitudinal sections through nodal diaphragms of *Phyllostachys nigra* shoot II. (A) Longitudinal section through the diaphragm of a node between internodes 6 and 7. The upper parenchyma cells (par-lacuna) will die and disappear, forming the lacuna. The sclerified parenchyma cells will form the border between the lacuna and the tissue of the diaphragm (par-border). (B) Longitudinal section through the diaphragm of a node between internodes 2 and 3 (culm sheath lost; internodes completely elongated). The cell walls are blue which indicates the presence of lignin. Ramification of xylem and phloem cells can be seen. Some vascular bundles have only one vessel bundle. (C) Longitudinal section through the diaphragm of the node between internodes 18 and 19 (culm sheath present; internodes not completely elongated). The cells are purple which indicates the absence of lignin. Both longitudinal and transverse-sectioned vascular bundles are present indicating that they run criss-cross in the nodal structure.
Par, Parenchyma; ph, phloem; scl, sclerenchyma sheath; xy, xylem.

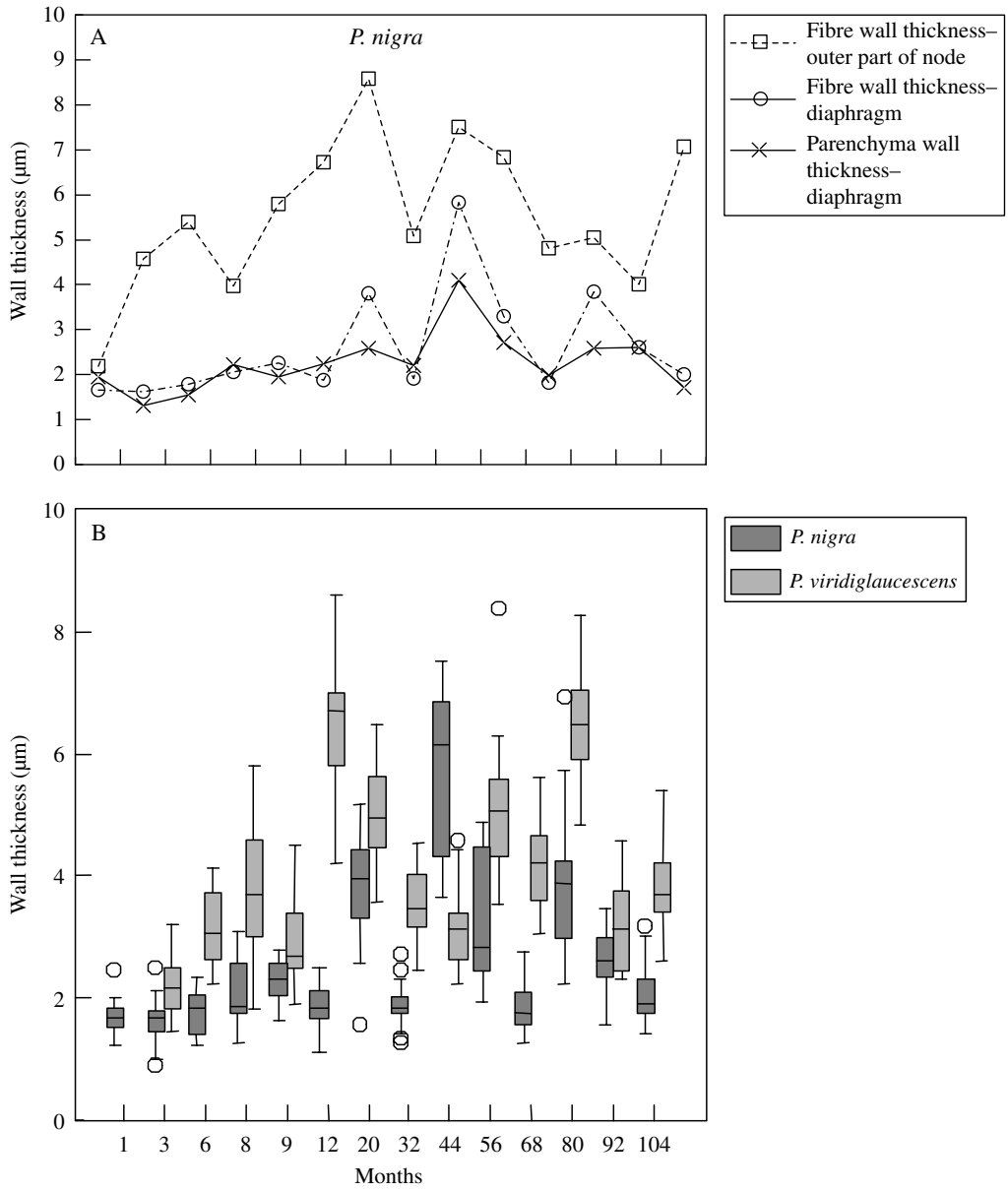



FIG. 3. Parenchyma and fibre wall thickness. (A) Comparison between parenchyma and fibre wall thickness of the outer part of the node and the nodal diaphragm. The cell walls are thinner in the nodal diaphragm. (B) Boxplots of fibre wall thickness in the diaphragm per age. Cell wall thickness does not significantly increase during ageing.

Fibre and parenchyma cell wall thickness is lower in the diaphragm than in the outer culm wall of the node. Nonetheless, the fibre diameter is higher in the nodal structure, which implies a potential for thicker cell walls in the diaphragm. The same observations are made in culms of *P. viridiglaucescens*. Boxplots (Fig. 3B) reveal an overlap between the measurements, indicating that age does not have a significant influence on cell wall thickness of both fibre and parenchyma cells in the nodal structure. It can be deduced that the values of *P. viridiglaucescens* are mostly higher than those of *P. nigra*. But again, due to the overlap of the boxes, this difference is not significant. It has to be noted that samples older than 12-months and the 8-month-old samples were harvested in the south of France, while the others are collected in Belgium. The difference in climate and soil could also have an impact.

### *UV absorbance spectra of individual cell wall layers of the tissue*

The cellular UV-spectra of the epidermis cell walls show a clear broad shoulder with absorbance maxima between 310 and 320 nm (Fig. 4A). This shoulder is typical for grass lignin and can be linked to the presence of *p*-coumaroylation as demonstrated by Higuchi (1987). There is a significant difference between nodes surrounded by culm sheaths and nodes without culm sheaths. Epidermis cells of nodes

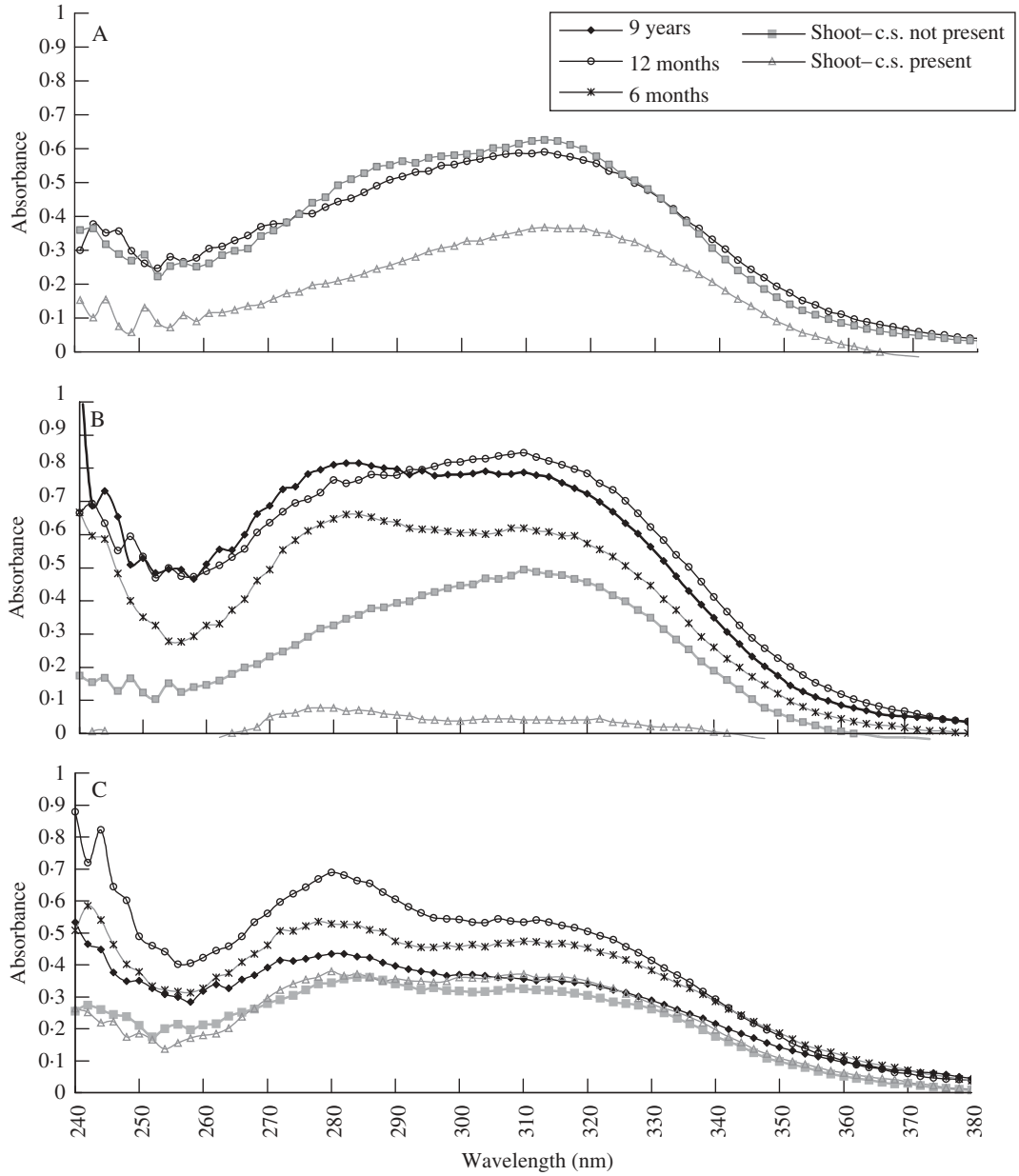



FIG. 4. UV-absorbance spectra: (A) UV-absorbance spectra of epidermal cell walls; (B) UV-absorbance spectra of hypodermal cells; (C) UV-absorbance spectra of cell wall layers of fibres at the outer culm wall.

protected by culm sheaths are less lignified, whereas epidermis cells of nodes without culm sheaths show similar absorbance behaviour in very young (shoots) and older (12-month-old) nodes.

Hypodermis cell walls have different lignin contents in samples of different ages (Fig. 4B). In nodes surrounded by culm sheaths the UV-spectra have relative low absorbance values. The spectra from nodes not surrounded by culm sheaths show a maximum absorbance at 310–320 nm, due to the esters of *p*-coumaric and ferulic acid. There is no shoulder at 280–282 nm, indicating the absence of the strongly absorbing guaiacyl lignin (Fergus and Goring, 1970; Musha and Goring, 1975). Older samples (6, 12 and 104 months old) have a higher lignin content characterized by spectra with a clearer peak at 280–282 nm and a shoulder between 310 and 320 nm. The absorbance values of a 6-month-old node are lower than those of 12- and 104-month-old nodes. The spectra of the young samples (shoots) have lower absorbance values compared with the absorbance values of the epidermis cells, whereas the older samples (12 and 104 months old) have higher values.

The UV spectra of $S_2$ walls of fibres at the outer culm wall all follow a similar pattern (Fig. 4C). There is no significant difference between the spectra of shoots (with and without culm sheaths) and of 6-, 12- and 104-month-old samples. The spectra curves have a clear peak at 280–282 nm and a broad shoulder at 310–320 nm. Compared with the epidermis and hypodermis cell walls, the ratio $abs_{310\ nm} : abs_{280\ nm}$

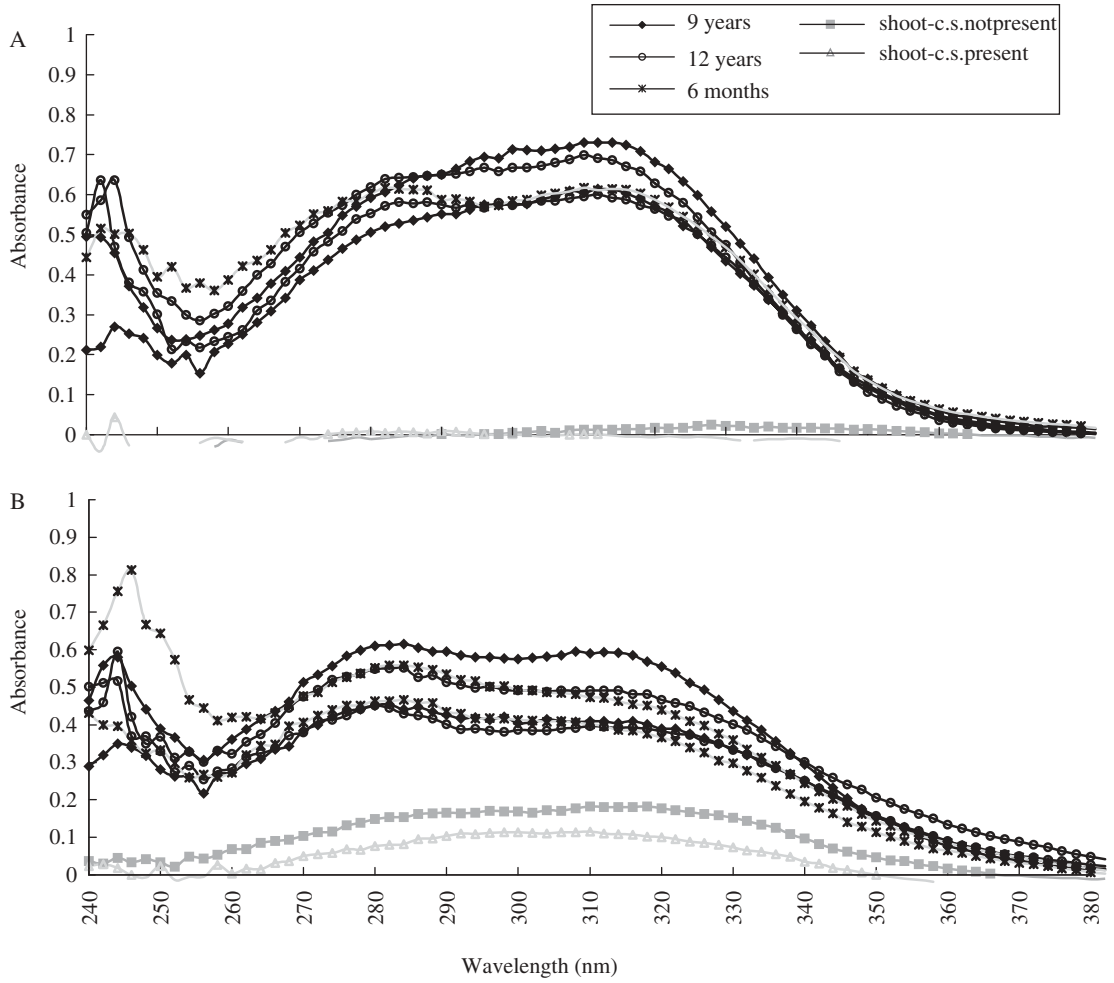



FIG. 5. UV-absorbance spectra: (A) UV-absorbance spectra of parenchyma cells in the diaphragm; (B) UV-absorbance spectra of cell wall layers of fibres in the diaphragm.

is lower. The absorbance values at 280 nm and 312 nm are significantly higher in the compound middle lamellae than in the $S_2$ wall layers (paired *t*-test, one-tailed; $P_{280\,nm} = 0{\cdot}042$; $P_{312\,nm} = 0{\cdot}023$). The spectra of the compound middle lamellae have a more clear shoulder at 310–320 nm, indicating the presence of more *p*-coumaric and ferulic acids in comparison to the $S_2$ wall layers.

Cell wall layers of parenchyma cells located in the diaphragm of very young nodes (shoots) are almost not lignified showing very low absorbance values without pronounced maxima (Fig. 5A). The UV-spectra of older nodes (6, 12 and 104 months old) exhibit similar absorbance values with a maximum (shoulder) between 310–320 nm. A second peak is present between 284 and 288 nm. Compared with the $S_2$ wall layers of fibres at the outer culm, this means a shift of absorption maximum from 280–282 nm toward longer wavelengths. The absorbance values are higher in the compound middle lamellae (paired *t*-test, one-tailed; $P_{280\,nm} = 0{\cdot}037$; $P_{312\,nm} = 0{\cdot}055$) but the spectra are similar to those of the cell walls. There is no significant (paired *t*-test, one-tailed; $P > 0{\cdot}05$) difference between the ratio $abs_{310\,nm} : abs_{280\,nm}$ in the $S_2$ wall layers and the compound middle lamellae.

The spectra curves of the fibres in the diaphragm of the young samples (shoots) do not have high absorbance values, although the UV absorbance of the fibres in the diaphragm of the shoots is higher compared with the UV absorbance of the parenchyma cells in the diaphragm of the shoots (Fig. 5B). The older nodes (6, 12 and 104 months old) show similar absorbance behaviour, with a peak at 280–282 nm and a shoulder between 310 and 320 nm. No shift of the absorbance maximum from 280–282 nm to longer wavelengths could be observed. The compound middle lamellae have approximately the same absorbance values for the young samples but higher absorbance values for the older samples (paired *t*-test, one-tailed; $P_{280\,nm} = 0{\cdot}033$; $P_{312\,nm} = 0{\cdot}02$). The ratio $abs_{310\,nm} : abs_{280\,nm}$ is the same in the $S_2$ wall layers and the compound middle lamellae of the fibres in the diaphragm (paired *t*-test, one-tailed; $P > 0{\cdot}05$).

### *Scanning profiles of different cell types of the tissue*

The colour pixels of the scanning profiles indicate different intensities of UV absorbance at $\lambda_{280\,nm}$. The high resolution ($0{\cdot}25\,\mu m^2$ per pixel) enables a high

differentiation of the UV absorbance within individual cell walls. Scanning profiles of epidermis and hypodermis cells of a node surrounded by a culm sheath and a node where the culm sheath is already lost, show a clear difference in lignin content within individual cell wall layers (Fig. 6A and B).

Fibres at the outer culm wall of older samples (6, 12 and 104 months old) show a lamellar structure with a decreasing lignin content towards the cell lumen. This lamellar structure can already be observed in a node without the protecting culm sheath at the outer culm wall, but not yet in fibres of nodes still surrounded by culm sheaths (Fig. 6C–E). The compound middle lamella and especially the cell corners are more lignified. Fibres in the diaphragm of a node without the protecting culm sheath have just started to form wall layers and to lignify them. Older samples already have fibres with cell wall layers with decreasing lignin content towards the cell lumen. A scanning profile of a longitudinal section of a fibre revealed that the middle and the end part of a fibre are equally lignified. Figure 6F illustrates xylem cells. Near the pits the lignin content is relatively low.

## DISCUSSION

### *Structure of the node*

In the nodes the structure is less organized than in the internodes. Since no radial conducting system exists in the internodes of bamboo culms because they are hollow, the transverse distribution of water and nutrients occurs mainly in the nodal region. Therefore, the typical composition of a vascular bundle in the internode is changed in the nodal region. In the node branching of the xylem and phloem can be observed. Ding and Liese (1997) studied the anatomy of bamboo nodes. They describe that at the branching point, the metaxylem consists of abundant small vessels, which may have more apertures. Between the vessels there are many small cells with pits resembling those of the vessels, but without perforations. The protoxylem vessels exhibit intensive branching and these structures facilitate the liquid movement in different directions. In the phloem, abundant lateral sieve areas enhance connection to sieve elements. A spindle-like glomerulus structure consisting of filiform elements connects phloem of the main vascular bundles to that of the branched ones (phloem ganglion). André (1998), who carried out microcasting of vascular bundles in the nodes of bamboo, gives a more complete picture of the vessel ramification. He stated that the vessels that ramify form cell clusters, which consist of many densely pitted vessel elements. This author demonstrated that the 'xylem transfer cells' (intensively pitted small cells lying between the vessels), as described by Zee (1974), and the 'cells derived from these xylem transfer cells', as described by Ding and Liese (1997), are in fact densely pitted vessel elements of the ramification-forming cell clusters. Also the transfer cells as described for wheat (Patrick, 1972; Busby and O'Brien, 1979) and other plant species (Gunning *et al*., 1970) are probably the vessel elements as described by André (1998). Ding *et al*. (2000) studied the development and ultrastructure of the phloem ganglion in the bamboo node. They distinguished two cell types, one with pointed ends but many pits in the lateral wall and another one at both ends of the spindle that possess an intermediate form between the filiform cell and the normal sieve tube. They state that the cells of the phloem ganglion have the character of transfer cells. Gunning *et al*. (1970) gives as definition of transfer cells: plant cells with wall ingrowths thereby increasing the area over which the plasma membrane is in contact with the extra-cytoplasmic environment and specialized in relation to short distance transport of solutes.

### *Cell wall thickening during ageing*

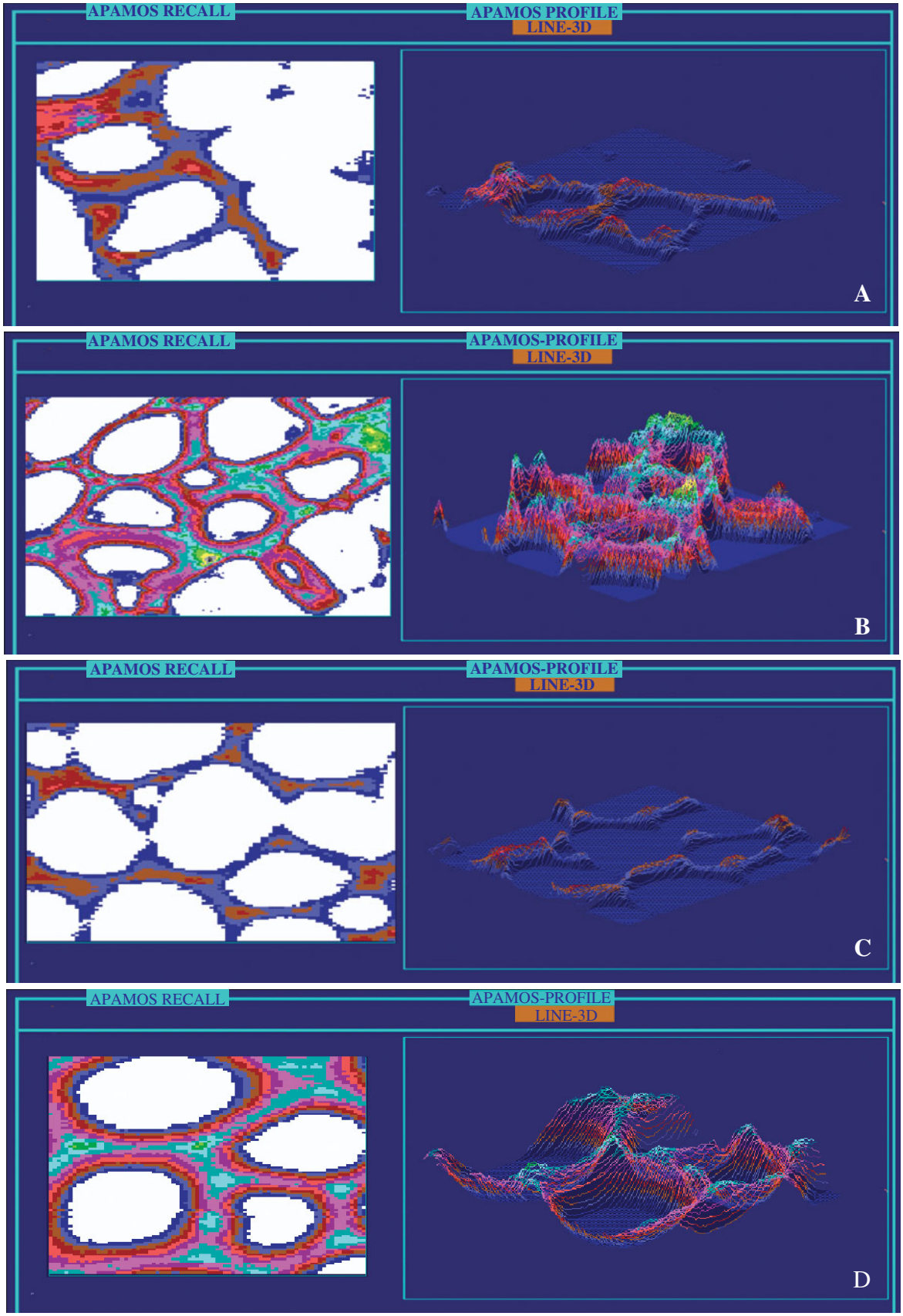
In the literature, no studies on cell wall thickening in the nodal structure of bamboos were found. This study reports that cell wall thickening does not significantly take place during ageing. In the diaphragm, cells with walls thinner than those in the outer part of the node, where the cell wall thicknesses are similar to those of the internode, are present. This can be explained by the fact that fibres in the outer part of the node probably surround vascular tissue passing directly from one internode to another and, as a result, have similar cell wall thicknesses to those in the adjacent internodes.

Not only thinner cell walls but also higher cell diameters were found in the diaphragm. Both elements indicate that the density is lower in the diaphragm. This can be related to the fact that nodes store energy to elastically restore stems after bending forces are removed; thick cell walls and high density would make the diaphragm too stiff to have this feature.

### *Lignin distribution and lignification during ageing*

Lybeer and Koch (2005) characterized UV-spectra of *P. viridiglaucescens* internodes as having an absorbance shoulder between 310–320 nm and a peak at 280–282 nm. The presence of a shoulder between 310 and 320 nm can be linked to the presence of *p*-coumaroylation as demonstrated by Higuchi (1987). An absorbance peak at 280–282 nm indicates the presence of the strongly absorbing guaiacyl lignin (Fergus and Goring, 1970; Musha and Goring, 1975).

Epidermis, hypodermis and fibre cell wall layers at the outer culm side of the node have similar spectra and absorbance values to cell wall layers of fibres at the outer culm side of the internode (Lybeer and Koch, 2005). Only the UV spectra of parenchyma and fibre cell wall layers of the diaphragm are different. In *P. viridiglaucescens* internodes, ferulic and *p*-coumaric acids are widely distributed and their content is dependent on the anatomical location and the differentiation phase. The epidermis cells and compound middle lamellae of fibres and parenchyma cells of bamboo internodes have high absorbance values (maxima) for the wavelengths related to these esters. Younger cell walls have higher ratios of $abs_{310\,nm} : abs_{280\,nm}$ than cell walls in older culms (Lybeer and Koch, 2005). In the nodes, hydroxycinnamic acids are even more widespread as they do not only occur in epidermis cells and compound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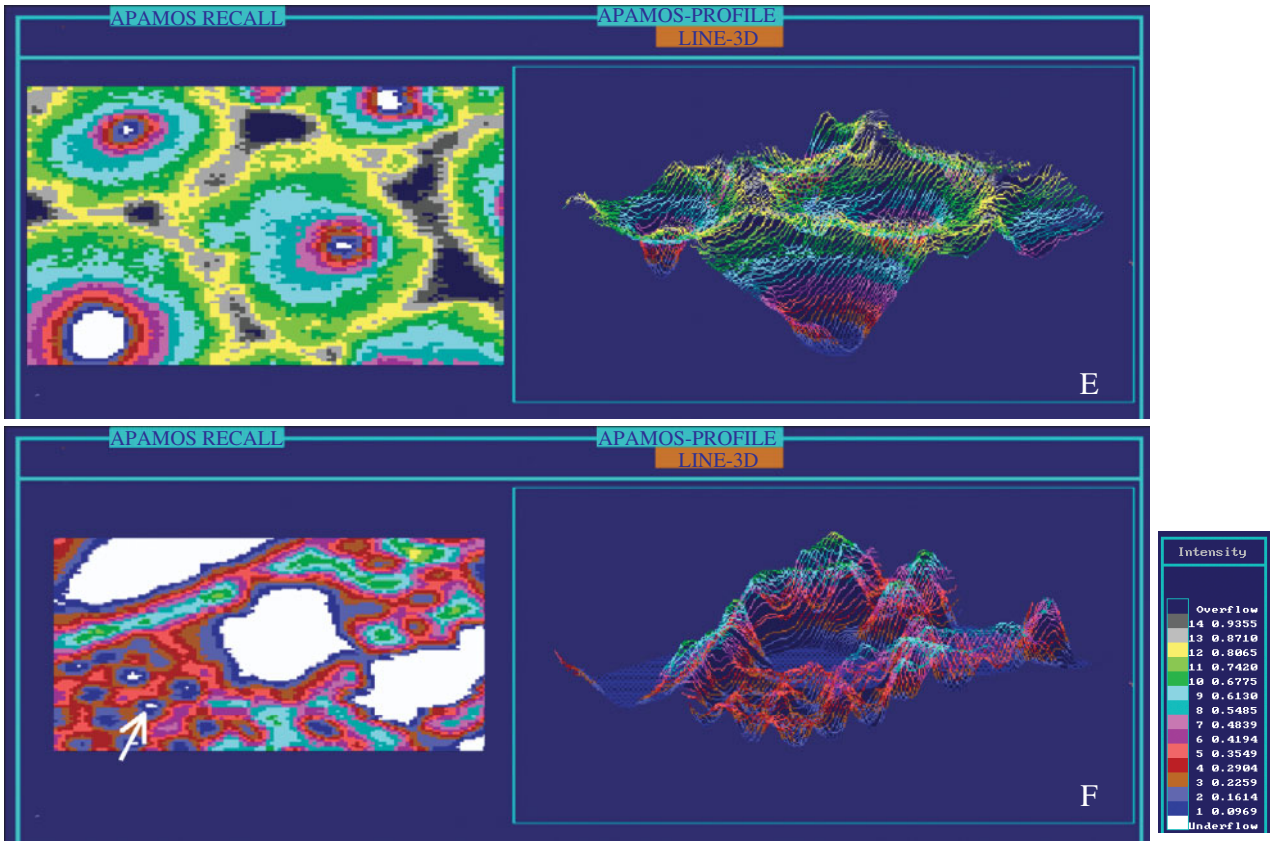


FIG. 6. UV-micrographs (left) and 3D profiles (right) of cell walls of *Phyllostachys nigra* (A–D) and *P. viridiglaucescens* (E and F). The colour pixels indicate the different UV-absorbance values at a wavelength of 280 nm. (A) Epidermis cells of a *P. nigra* shoot without a culm sheath; (B) epidermis cells of a *P. nigra* shoot with a culm sheath; (C) outer fibres of a *P. nigra* shoot without a culm sheath; (D) outer fibres of a *P. nigra* shoot with a culm sheath; (E) outer fibres of a 104-month-old *P. viridiglaucescens* culm; (F) xylem cells of a *P. viridiglaucescens* culm of 12 months old (the arrow points to pits).

middle lamella, but also in parenchyma and fibre cell wall layers of the diaphragm of older culms. Hydroxycinnamic acid moieties cause the cross-linkage of cell-wall polysaccharides and participate with lignin to generate polysaccharide–lignin complexes, which lead to an increase in wall rigidity (Ishii, 1997; Morrison *et al*., 1998). Hydroxycinnamic acids thus have an important effect on the wall mechanical properties. Ferulic acid esters act as lignin initiation sites and direct cell wall cross-linking during plant growth and development. The role of *p*-coumaric acid occurs later and serves to bind together the growing predominant syringyl lignin polymer (Morrison *et al*., 1998). Morrison *et al*. (1998) studying the cell wall composition of maize internodes of varying maturity concluded that rind tissue (cuticle, epidermis, xylem elements and phloem) of maize generally has greater ferulic acid and *p*-coumaric acid ester concentrations than pith tissue (parenchyma cells and randomly distributed vascular strands). As ferulic acid esters act as lignin initiation sites and direct cell-wall cross linking during plant growth and development, this was expected because rind vascular tissues lignify to a greater extent to support conductive and supportive tissue of the internode. Differences between pith and rind concentrations of ferulic acid esters diminished as internode tissues further differentiated and added cell-wall components. Nodes have an important mechanical function for the bamboo culm as they act as braces to resist wall invagination or buckling and as they increase the effective stiffness of stems (Niklas, 1997, 1998). This could be a possible explanation for the higher presence of hydroxycinnamic acid moieties in the nodal diaphragm as hydroxycinnamic acids increase the wall rigidity due to cross-linking with cell wall polysaccharides. This increase in wall rigidity by generating polysaccharide–lignin complexes perhaps increases the stiffness more than the deposition of guaiacyl units with lower linking. Furthermore, as thick cell walls and high density would make the diaphragm too stiff to elastically restore stems after bending forces are removed, the cell walls themselves need to give enough support to resist to mechanical stresses.

He and Terashima (1991) found a shift from 280 nm toward longer wavelengths and from 310–320 nm toward shorter wavelengths with the progress of lignification (i.e. at later differentiation stages) in the cell corners of fibres in rice (*Oryza sativa* L.) and sugarcane (*Saccharum officinarum* L.). They worked out that this shift is due to the increase in the total content of hydroxycinnamic acid esters. A similar shift from 280–282 nm toward longer wavelengths could be observed in the parenchyma cells of the diaphragm but not in other cells.

There is a clear difference in lignin content between cells of shoots and of older culms. This difference is especially

clear in the fibre and parenchyma cells of the diaphragm, which illustrates that the lignification process starts at the outside and proceeds towards the centre of the diaphragm. In the epidermis and hypodermis cells, shoots with a culm sheath have a lower lignin content than shoots without a culm sheath. This is probably because the hard structure of the culm sheath protects the weak, less-lignified underlying structures. Significant differences in lignin content between 6-month-old, 12-month-old and 9-year-old culms could not be observed. Lybeer and Koch (2005) demonstrated the same situation in internodes of *P. viridiglaucescens*. Their results are in contrast with the conclusions of several authors (Murphy and Alvin, 1997*a*; Lin *et al*., 2002) but agree with the findings of Itoh (1990) who stated that lignification is completed within one growing season. However, the authors mention that it is important to keep in mind that the spectra represent only one of several layers of a cell wall. It could be possible that lignification is completed within one cell wall layer while the fibre retains the ability to form new layers and lignify them additionally. The scanning profiles showed a lamellar structure of fibre cells with a decreasing lignin content towards the cell lumen.

The xylem transfer cell wall has a low lignin content. This is in contrast with most dicotyledons (Musha and Goring, 1975; Saka and Goring, 1988) and the monocotyledon *Triticum aestivum* L. (Donaldson *et al*., 2001) but agrees with the findings of a low lignin content in bamboo vessel cell walls in the internode (Lybeer and Koch, 2005). Near the pits there is only a low lignin content.

## CONCLUSIONS

Nodes are not only important for the lateral transport of water and nutrients within the culm and between the culm, the branches and the leaves but they also have a major mechanical function. During development, the hard culm sheath protects the weak, non-lignified structures. In a fully elongated stem, the nodal structure can act as a spring-like joint to support the culm by bending forces as is demonstrated by Niklas (1998). This function is reflected in its anatomy. The combination between on the one hand larger diameters and thinner cell walls and on the other hand presence of more hydroxycinnamic acid moieties in the diaphragm makes it a flexible but strong structure.

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